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Thieno[3,2-*c*]pyrazoles: A novel class of Aurora inhibitors with favorable antitumor activity

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ABSTRACT

A novel series of 3-amino-1*H*-thieno[3,2-*c*]pyrazole derivatives demonstrating high potency in inhibiting Aurora kinases was developed. Here we describe the synthesis and a preliminary structure–activity relationship, which led to the discovery of a representative compound (**38**), which showed low nanomolar inhibitory activity in the anti-proliferation assay and was able to block the cell cycle in HCT-116 cell line. This compound demonstrated favorable pharmacokinetic properties and good efficacy in the HL-60 xenograft tumor model.

1. Introduction

Targeting mitosis is a well known approach in the search for anti-cancer therapies. For example, the taxanes and vinca alkaloids, some of the most effective compounds in the oncology field, are anti-mitotics. Unfortunately the collateral effects related to their activity on microtubules (e.g., neurotoxicity) still remain an unsolved issue. The search for well tolerated anti-mitotic agents continues to represent a challenge in drug discovery. Within the family of Ser/Thr protein kinases, we consider mammalian Aurora kinases, which secure the correct progression of cell cycle during mitosis or meiosis,[1,2] as biological targets worth pursuing in the quest for new therapeutic agents in the oncology field. More than 10 Aurora kinase inhibitors are currently under clinical evaluation.[3] At Nerviano Medical Sciences we have been actively contributing to this field: Danusertib (PHA-739358), one of the most advanced compounds in clinic,[4] pioneered Aurora inhibition in patients. The same compound also showed activity on mutated forms of Bcr-Abl. Among those the T315I mutation is the major cause of resistance to Imatinib, as well as to other Bcr-Abl inhibitors of second generation.[5]

Our chemical expansion of 3-amino-tetrahydropyrrolo[3,4-*c*]pyrazole **1**,[6] a versatile scaffold designed to target the ATP pocket of protein kinases, led to the identification of PHA-739358.[7,8] The initial goal, that is, to obtain novelty, multiple diversity points and amenability for combinatorial expansion by incorporating a 3-aminopyrazole moiety within a bicyclic heterocycle, was therefore achieved and laid the ground for subsequent optimizations to a clinical candidate. Following this strategy we designed several novel templates for kinase inhibition (Chart 1), including 3-amino-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid **2**,[9–11] 3-amino-1

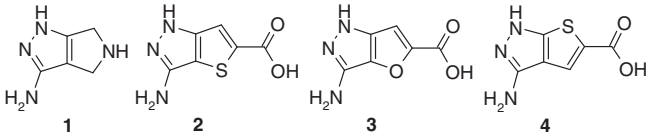

Chart 1.

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H-furo[3,2-*c*]pyrazole-5-carboxylic acid **3**,[9–11] and 3-amino-1*H*-thieno[2,3-*c*]pyrazole-5-carboxylic acid **4**.[12,13] Here we report on the optimization of the inhibitory activity of the thieno[3,2-*c*]pyrazole series resulting in the identification of a promising lead compound endowed with high potency against Aurora kinases in vitro and in the HL-60 xenograft tumor model in vivo.

2. Chemistry

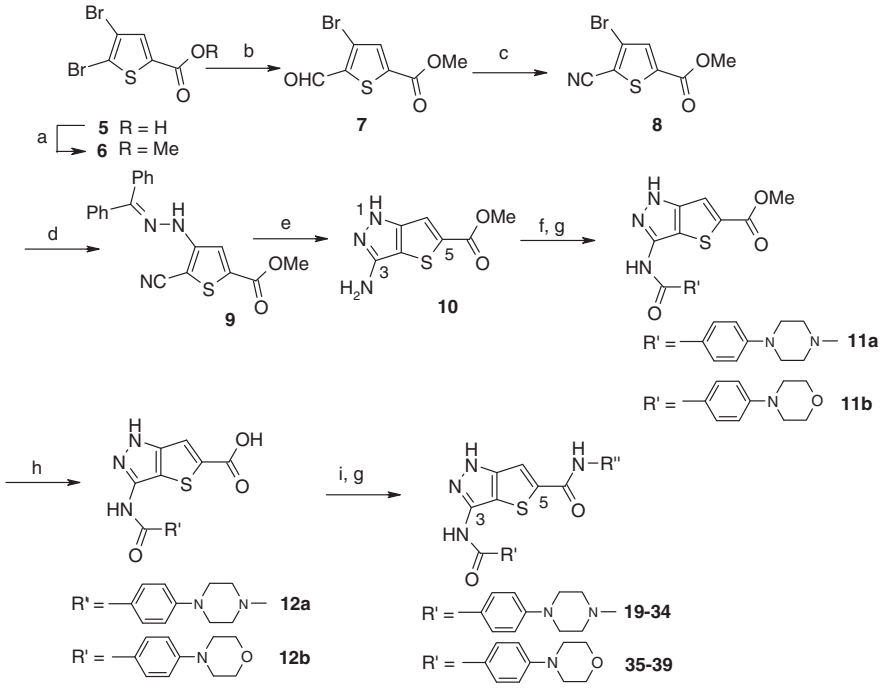
The synthetic pathway to methyl 3-amino-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylate **10** and target compounds **19**–**39** is outlined in Scheme 1. The 4,5-dibromo-thiophene-2-carboxylic acid methyl ester **6** was obtained from commercially available 4,5-dibromo-thiophene-2-carboxylic acid **5** by treatment with methanol and sulfuric acid, at reflux temperature. The formylation was performed exploiting an efficient halogen–Mg exchange with *i*-PrMgCl[14a,b] and a subsequent reaction with DMF to obtain 4-bromo-5-formyl-thiophene-2-carboxylic acid methyl ester **7**. Next, compound **7** was converted to the corresponding oxime by treatment with hydroxylamine hydrochloride and dehydrated with trifluoroacetic anhydride[15] to give the 5-cyano-derivative **8**. The reaction of benzophenone hydrazone with compound **8** in toluene at 100 °C using palladium acetate (3 mol %) and 1,1′-bis(diphenylphosphino)ferrocene (DPPF) (6 mol %) as catalytic system[16] gave 4-(*N*′-benzhydrylidene-hydrazino)-5-cyano-thiophene-2-carboxylic acid methyl ester **9**. Treatment of **9** with hydrochloric acid efficiently gave the condensed amino pyrazole nucleus.[17] Thus, the desired scaffold 3-amino-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester **10** was produced along with its corresponding acid (30%), which was reconverted to its methyl ester by treatment of the crude residue with methanol and sulfuric acid at reflux.

The subsequent class expansion was carried out by synthesizing different amides at positions 3 and 5. Reaction of compound **10** with an excess of acyl halide in DCM led to the 1,3-bisacyl-derivatives, which were easily converted to the 3-acylamino-derivatives **11** by treatment with TEA/MeOH. Finally, two methods were developed, which allowed the synthesis of different amides at position 5, either by individual solution-phase synthesis (Scheme 1) or by parallel solid-phase chemistry (Scheme 2). In solution, standard alkaline hydrolysis led to the carboxylic acids **12a** and **12b**. These were activated by treatment with EtOCOCl and coupled with different amines to give the 1-carbethoxy-5-amido-derivatives, which were converted into the target compounds **19**–**39** by hydrolysis with TEA/MeOH. Alternatively **11** was loaded on polystyrene trityl resin and the resulting resin bound protected thienopyrazoles **13** were hydrolyzed with NaOH to afford the corresponding carboxylic acids **14**. Coupling with amines in the presence of TBTU and DIPEA furnished the 5-amido-derivatives **15**, which were cleaved from the resin using 40% TFA/DCM to give target compounds **16**. The library design was defined by the combination of 6 acyl substituents in 3-position (step a, Scheme 2) with 48 amines in 5-position (step e, Scheme 2) to lead to a set of 256 compounds. The synthesis of the intermediates **13** (step c, Scheme 2) were carried out using 10 g of polystyrene tritylchloride resin, while intermediates **14** were divided in aliquots of 200 mg of resin to obtain about 10–20 mg of final, HPLC purified compounds **16**.

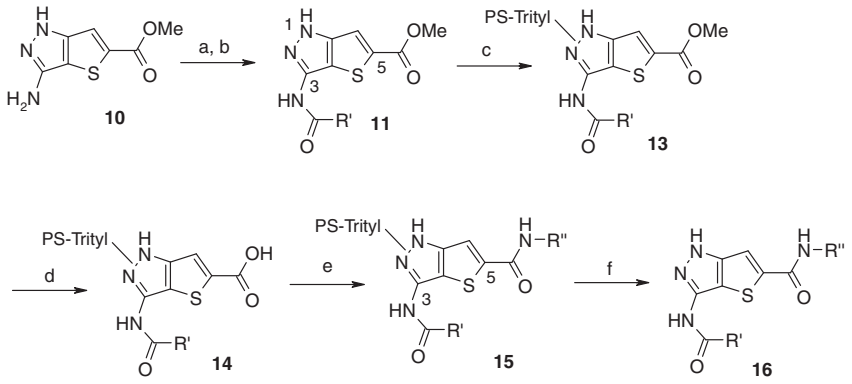
3. Results and discussion

During the development of the structurally related pyrrolopyrazole Aurora inhibitors,[6,7] benzoic amides substituted in para position with tertiary amines emerged as the optimal substituents at position 3, leading to compounds endowed with high potency in biochemical and cellular assays as well as acceptable aqueous solubility. Based on this result, we initially focused the expansion of the thieno[3,2-*c*]pyrazoles on derivatives bearing a 4-(4-methyl-piperazin-1-yl) or a 4-morpholin-4-ylbenzamide at position 3 (Table 1).

The resulting compounds were tested for their ability to inhibit Aurora-A in vitro and their anti-proliferative activity was evaluated

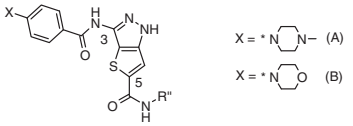


Scheme 1. Solution-phase synthesis of compounds **19**–**39**. Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 7 h; (b) *i*-PrMgCl, DMF, −35 to 22 °C, 3 h; (c) NH_2OH HCl, pyridine, CH_3CN, 1 h, then $(CF_3CO)_2O$, 3 h; (d) $(C_6H_5)_2C{=}NNH_2$, $Pd(CH_3CO_2)_2$ (3 mol %), DPPF (6 mol %), Cs_2CO_3, toluene, 100 °C, 16 h; (e) HCl, MeOH/THF, reflux, 10 h; H_2SO_4, MeOH, reflux, 17 h; (f) R′COCl (3.5 equiv), pyridine, DCM, 22 °C, 12 h; (g) 10% TEA/MeOH, 50 °C, 4 h; (h) 2 M NaOH/MeOH, 55 °C, 16 h; (i) H_2NR'', EtOCOCl, DIPEA, DMF, 22 °C, 16 h.



Scheme 2. Solid-phase synthesis of compounds **16**. Reagents and conditions: (a) R′COCl (3.5 equiv), pyridine, DCM, 22 °C, 12 h; (b) 10%TEA/MeOH, 50 °C, 4 h; (c) PS-TritylCl, DIPEA, DCM/DMF, 22 °C, 16 h; (d) NaOH (30 equiv), THF/H_2O/MeOH, 22 °C, 72 h; (e) H_2NR'', TBTU, DIPEA, DCM, 22 °C, 16 h; (f) 40% TFA/DCM, 22 °C, 1 h.

Table 1
Aurora-A inhibition of representative 1*H*-thieno[3,2-*c*]pyrazoles



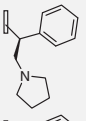
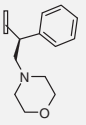
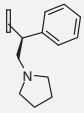
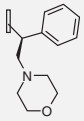
Compd	X	R″	Aur-A[a,c]	HCT-116[b,c]	Solubility[d]
19	A	Et	0.048	0.71	67
20	A	*i*Pr	0.059	0.82	59
21	A	Ph	0.105	na	6
22	A	Bn	0.032	0.31	8
23	A		0.013	0.032	6
24	A		0.010	0.013	1
25	A		0.048	0.09	<1
26	A		0.006	0.02	<1
27	A		0.066	0.20	<1
28	A		0.009	0.08	<1
29	A		0.031	na	<1
30	A		0.044	0.035	2
31	A		0.052	1.40	<1
32	A		0.045	na	<1
33	A		0.027	0.040	93
34	A		0.018	0.037	15
35	B	Bn	0.011	0.23	<1

Table 1 (*continued*)

Compd	X	R″	Aur-A[a,c]	HCT-116[b,c]	Solubility[d]
36	B		0.005	0.08	<1
37	B		0.083	2.17	<1
38	B		0.018	0.022	85
39	B		0.025	0.15	2

[a] Enzyme inhibition IC_{50} μM.
[b] Antiproliferation IC_{50} μM.
[c] Values are mean from two or more independent dose–response curves; variation was generally ±25%.
[d] μM, pH 7. Values are means of two or more experiments.

on the human colon carcinoma cell line HCT-116. A limited preliminary SAR exploration (**19**–**25**) indicated benzylamides, preferably substituted by small alkyl groups in alpha position, as the best substituents at position 5. The Pro-R configuration was preferred and resulted in high inhibitory potency and anti-proliferative activity (**26** vs **27** and **28** vs **29**). Gem di-methyl substitution is associated with good potency (**30**), whilst cyclic analogs are less active in the cellular assays (**31**). Unfortunately the most potent compounds in this set (**26**, **28**, **30**) showed limited aqueous solubility. In order to better understand the SAR and to potentially identify sites for attaching solubilizing groups, the crystal structure of Aurora-A in complex with compound **28** was solved. As shown in Figure 1, the structure revealed that the phenyl group is directed under the glycine-rich loop and the ethyl group points down towards the main chain of residues Glu260-Asn261 at the bottom of the pocket. Furthermore, the ethyl group sits in a relatively wide cavity that starts next to the side chain of Leu263 and extends towards the side chain of residues Glu260, Asn261 and Asp274. Thus, solubilizing groups that take advantage of this cavity could be added to the scaffold. Indeed, by placing a second tertiary amino group on the molecule it is possible to reach an acceptable aqueous solubility (**33**, **34**) while maintaining high potency. The key compounds were replicated in the 4-morpholin-4-yl-benzamido series and confirmed the SAR trend (**35**–**39**) outlined so far. From this survey, **33** and **38** emerged as the most soluble compounds. The

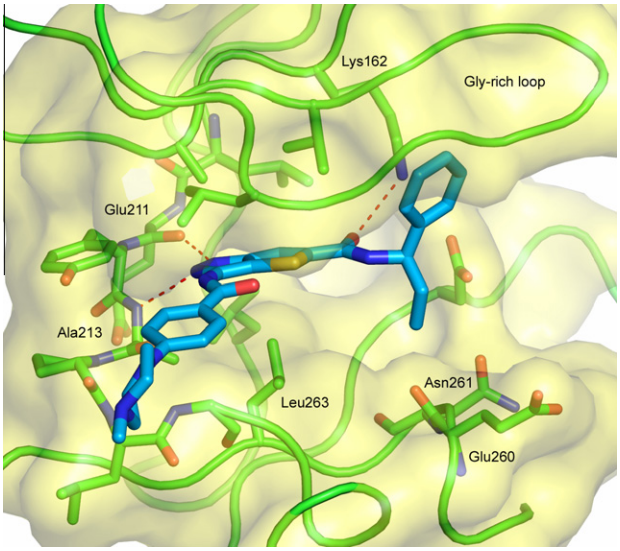


Figure 1. View of compound **28** in the active site of Aurora-A.

latter, more active in both in vitro assays, was selected for a deeper evaluation.

Screening of compound **38** against a panel of 40 kinases representing diverse families of Tyr and Ser-Thr kinases revealed it to be a potent pan Aurora inhibitor with activities ranging from 18 nM (Aurora A) to 62 nM (Aurora C). Furthermore the cross reactivity with FGFR1, VEGFR3, KIT (IC_{50} = 14, 19 35 nM, respectively) is not expected to alter the cell cycle profile and the cellular phenotype typical of an Aurora inhibitor.[8] (Experimental details are available online in Supplementary data.)

As expected and described for other Aurora inhibitors,[6,8] the incubation of HCT-116 cells for 24 h with compound **38** (as the bismethanesulfonate salt) at different concentrations led to an accumulation of cells with ⩾4 N DNA content (Fig. 2). Furthermore compound **38** is able to inhibit histone H3 phosphorylation on Ser10 in HCT-116 cells (Fig. 3). This is consistent with Aurora-B inhibition, as widely described for Aurora kinase inhibitors. Compound **38** demonstrated potent anti-proliferative activity (IC_{50} values ranging from 7 to 149 nM, Table 2) in several tumor cell lines,

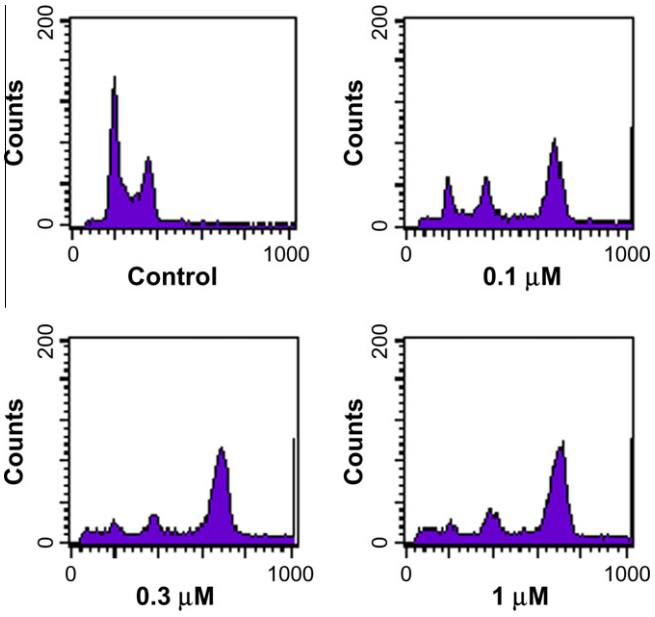


Figure 2. Flow cytometric analysis of DNA content in human colon carcinoma cells (HCT-116) treated for 24 h with increasing concentration of **38**.

Table 2
Inhibition of cell proliferation by compound **38**

Cell line	IC_{50}[a] (μM)
DU-145	0.149
HT-29	0.065
PC-3	0.057
HCT-116	0.022
MCF-7	0.014
A-2780	0.013
Colo205	0.007

[a] Values are the mean from two or more independent dose–response curves; variation was generally ±25%.

Table 3
Individual and average pharmacokinetic parameters estimated in male CD-1 mice following IV bolus (the second dose of a IV bid treatment 12 h apart) at the nominal dose of 10 mg/kg/bid with compound **38**

Parameters	M1	M2	M3	Mean	SD
C_{max} (μM)	9	8	11	9	1
$t_{1/2}$ (h)	7	6	7	7	0.4
AUC daily (μM h)	10	7	9	9	1
CL (L/h/kg)	3	4	3	3	0.4
V_{ss} (L/kg)	17	16	14	16	2

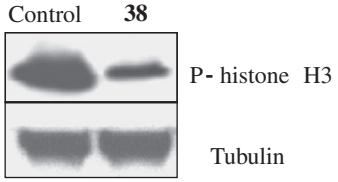


Figure 3. Phosphorylation of histone H3 after treatment of HCT-116 cells for 24 h with 1 μM compound **38**. Normalization of the loaded protein was done using an antibody which recognizes Tubulin.

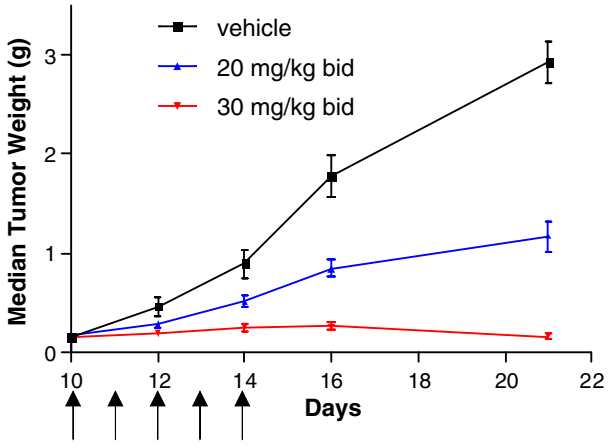


Figure 4. Antitumor activity of **38** against HL-60 human leukemia xenograft implanted in SCID mice. The TGI (%) was calculated according to the equation %TGI = 100 − (mean tumor weight of treated group/mean tumor weight of control group × 100). Eight animals/group. Arrows indicate dosing (bid × 5).

including colon carcinoma HCT-116, HT-29 and Colo205, ovarian carcinoma A2780, breast carcinoma MCF7 and prostate carcinoma DU-145 and PC-3.

A pharmacokinetic profile of compound **38** was obtained after iv administration at a dose of 10 mg/kg in male CD-1 mice (Table 3). Compound **38** displayed high clearance (3 L/h/kg) and also a volume of distribution higher than the total body water,

indicating extensive tissue distribution. Plasma levels were detectable up to 24 h post dosing, having an average terminal half-life of about 7 h and an AUC of 9 μM h. When tested orally **38** showed negligible oral bioavailability (data not shown).

An antitumor activity of **38** was observed when the compound was administered to mice bearing the HL-60 human acute myelogenous leukemia xenograft model. As shown in Figure 4 the compound, given iv bid for five days at 20 and 30 mg/kg, showed a good dose dependency and a tumor growth inhibition of up to 94% (30 mg/kg, day 21) with an increase in body weight loss at the higher dose (29% at day 21).

4. Conclusion

A new aminopyrazole-based scaffold, useful for kinase inhibition, has been prepared and efficiently explored via homogeneous and solid phase synthesis. The optimization of thieno[3,2-*c*]pyrazoles towards Aurora inhibition led to the identification of compound **38**, a potent inhibitor able to block cell cycle and tumor cell proliferation in vitro in the nanomolar range. Compound **38** is efficacious in vivo in the HL-60 tumor model and is able to induce significant TGI. This study exemplifies another successful approach of the development of bicyclic pyrazole scaffolds and anticipates additional achievements in the area of similar heterocycles, such as the expansion on a regioisomeric scaffold and on analogous furopyrazoles (**4** and **3**, respectively, Chart 1), to be reported in future publications.

5. Experimental

5.1. Chemistry

All solvents and reagents, unless otherwise stated, were commercially available, of the best grade and were used without further purification. All experiments dealing with moisture-sensitive compounds were conducted under dry nitrogen or argon. Organic solutions were evaporated using a Heidolph WB 2001 rotary evaporator at 15–20 mmHg. Thin-layer chromatography was performed on Merck Silica Gel 60 F_{254} pre-coated plates. Column chromatography was conducted either under medium pressure on silica (Merck silica gel 40–63 μm) or on prepacked silica gel cartridges (Biotage). Components were visualized by UV light (λ: 254 nm) and by iodine vapor. ^{1}H NMR spectra were recorded at a constant temperature of 28 °C on a Varian INOVA 400 spectrometer operating at 400.45 MHz and equipped with a 5 mm Indirect Detection PFG Probe (^{1}H{^{15}N–^{31}P}). Chemical shifts were referenced with respect to the residual solvent signals (DMSO-d_6: 2.50 ppm for ^{1}H). Data are reported as follows: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br s = broad singlet, td = triplet of doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet), coupling constants (Hz), and number of protons. Electrospray (ESI) mass spectra were obtained on a LCQ ion trap (ThermoFinnigan). HPLC–UV–MS analyses, used to assess compound purity, were carried out combining the ion trap MS instrument with HPLC system SSP4000 (Thermo Separation Products) equipped with an autosampler LC Pal (CTC Analytics) and UV6000LP diode array detector (UV detection 215–400 nm). Instrument control, data acquisition and processing were performed by using XCALIBUR 1.2 software (ThermoFinnigan). HPLC chromatography was run at room temperature, and 1 mL/min flow rate, using a Waters X-Terra RP 18 column (4.6 × 50 mm; 3.5 μm). Mobile phase A was ammonium acetate 5 mM buffer (pH 5.5 with acetic acid)/acetonitrile 90:10, and mobile phase B was ammonium acetate 5 mM buffer (pH 5.5 with acetic acid)/acetonitrile 10:90; the gradient was from 0% to 100% B in 7 min then hold 100% B for 2 min before equilibration. ESI(+) high resolution mass spectra (HRMS) were obtained on a Waters Q-Tof Ultima directly connected with micro HPLC 1100 Agilent as previously described.[18] Elemental analyses were performed on a Carlo Erba 1110 instrument, and C, H, and N results were within ±0.4% of theoretical values unless specified.

5.1.1. General procedure A

General procedure for the preparation of compounds **12a**–**12b** is illustrated below for the preparation of **12a**.

5.1.1.1. 4,5-Dibromo-thiophene-2-carboxylic acid methyl ester (6). 98% H_2SO_4 (16 mL, 0.299 mol) was added drop wise to a suspension of 4,5-dibromo-thiophene-2-carboxylic acid (24.5 g, 0.085 mol) in methanol (250 mL). The reaction mixture was refluxed for 7 h, then the solvent was evaporated under vacuum and the residue diluted with 20% NaOH until pH 8. The aqueous layer was extracted with DCM (3 × 200 mL) and the separated organic phase was dried over sodium sulfate. The solvent was evaporated to dryness to give the title compound **6** as a white solid (24.3 g, 95%), which was used in the next step without further purification.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (s, 1H), 3.85 (s, 3H); LC–MS (ESI) *m/z* 299 [M+H]$^+$.

5.1.1.2. 4-Bromo-5-formyl-thiophene-2-carboxylic acid methyl ester (7). 2 M *i*-PrMgCl in THF (44.5 mL, 0.089 mol) was slowly added to a solution of **6** (24.3 g, 0.081 mol) in dry THF (200 mL) at −35 °C. The reaction was kept at the same temperature for 2 h, DMF (18.8 mL, 0.243 mol) was added and then allowed to reach room temperature. The reaction was stirred for 3 h and then poured into a mixture of 1 M HCl (200 mL) and MTBE (250 mL); the organic layer was separated and the aqueous layer washed with MTBE (1 × 200 mL). The re-collected organic fractions were dried over sodium sulfate and the solvent evaporated to dryness. The obtained residue was suspended in *n*-hexane (90 mL), stirred for 3 h then filtered to give the title compound **7** as a light brown solid (16.5 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.01 (s, 1H), 3.91 (s, 3H); LC–MS (ESI) *m/z* 249 [M+H]$^+$.

5.1.1.3. 4-Bromo-5-cyano-thiophene-2-carboxylic acid methyl ester (8). Hydroxylamine dihydrochloride (5.0 g, 0.073 mol) was added to a solution of **7** (16.5 g, 0.066 mol) in anhydrous acetonitrile (160 mL). Pyridine (32 mL, 0.397 mol) was added and after 1 h, cooling at 10–15 °C, trifluoroacetic anhydride was added drop wise. The mixture was stirred at room temperature for 3 h and then poured into a mixture of 1 M HCl (130 mL), H_2O (100 mL) and ethyl acetate (170 mL); the organic layer was separated and the aqueous layer extracted with ethyl acetate (1 × 150 mL). The re-collected organic fractions were dried over sodium sulfate and the solvent evaporated to dryness. The residue was purified by flash-chromatography (MTBE/cyclohexane 1:9) to give the title compound **8** as a white solid (14 g, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, 1H), 3.91 (s, 3H); LC–MS (ESI) *m/z* 246 [M+H]$^+$.

5.1.1.4. 4-(*N*′-Benzhydrylidene-hydrazino)-5-cyano-thiophene-2-carboxylic acid methyl ester (9). A solution of **8** (13.7 g, 0.055 mol) and benzophenone hydrazone (13.1 g, 0.066 mol) in dry toluene (500 mL) was added, under argon, to a suspension of DPPF (1.85 g, 0.0033 mol), Pd $(CH_3CO_2)_2$ (0.375 g, 0.0017 mol) and Cs_2CO_3 (27.2 g, 0.083 mol) in dry toluene (70 mL). The mixture was heated to 100 °C for 16 h, allowed to cool to room temperature and then filtered. The filtrate was evaporated to dryness to give 24.7 g of a solid, which was purified by flash-chromatography (ethyl acetate/cyclohexane 2:8). The collected fractions were evaporated to dryness and the resulting solid was taken up with ethyl acetate and filtered to give the title compound **9** as a yellow solid

(19 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 7.7–7.3 (m, 11H), 3.85 (s, 3H); LC–MS (ESI) *m/z* 362 $[M+H]^+$.

5.1.1.5. 3-Amino-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester (10). 37% HCl (150 mL 0.420 mol) was added drop wise to a solution of **9** (19.0 g, 0.052 mol) in MeOH (190 mL)/THF (150 mL). The reaction mixture was refluxed for 10 h, then was concentrated and filtered to give 8.4 g of the title compound in mixture with the corresponding carboxylic acid. 98% H_2SO_4 (3.6 mL, 0.065 mol) was added drop wise to a suspension of this solid in methanol (100 mL), and refluxed for 17 h. The mixture was concentrated and the residue was diluted with water (90 mL). 2-Dimethylethanol (9 mL, 0.089 mol) was slowly added (pH 7–8) and the resulting mixture was filtered to afford the title compound **10** as light yellow solid (6.30 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 7.59 (s, 1H), 5.32 (s, 2H), 3.85 (s, 3H); LC–MS (ESI) *m/z* 198 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_7H_7N_3O_2S + H^+$ 198.0332, found 198.0333.

5.1.1.6. 3-[4-(4-Methyl-piperazin-1-yl)benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester (11a). Oxalyl chloride (38.3 mL, 0.438 mol) was added to a suspension of 4-(4-methyl-piperazin-1-yl)-benzoic acid (16.1 g, 0.073 mol) in DCM (400 mL) and DMF (0.64 mL). After refluxing the mixture for 5 h, volatiles were removed under reduced pressure. The resulting 4-(4-methyl-piperazin-1-yl)-benzoyl chloride di-hydrochloride was added portion wise to a solution of **10** (4.0 g, 0.020 mol) in dry DCM (480 mL) and pyridine (24 mL, 0.304 mol). The suspension was stirred 16 h at room temperature. After solvent removal, the residue was dissolved in MeOH (300 mL) and Et_3N (30 mL) and stirred at 50 °C for 6 h. The solvent was evaporated and the solid crystallized from methanol to give the title compound **11a** as a white powder (4.6 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (s, 1H), 11.06 (s, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.69 (s, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.87 (s, 3H), 3.24–3.44 (m, 4H), 2.42–2.58 (m, 4H); 2.26 (br s, 3H); LC–MS (ESI) *m/z* 400 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{19}H_{21}N_5O_3S + H^+$ 400.1438, found 400.1440.

5.1.1.7. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester (11b). By employment of the above-described procedure, using 4-morpholin-4-yl-benzoic acid, compound **11b** was prepared. Yield, 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (s, 1H), 11.08 (s, 1H), 8.00 (d, *J* = 9.0 Hz, 2H), 7.68 (s, 1H), 7.02 (d, *J* = 9.0 Hz, 2H), 3.86 (s, 3H), 3.73–3.77 (m, 4H), 3.25–3.30 (m, 4H); LC–MS (ESI) *m/z* 387 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{18}H_{18}N_4O_4S + H^+$ 387.1122, found 387.1124.

5.1.1.8. 3-[4-(4-Methyl-piperazin-1-yl)benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (12a). A 2 N solution of NaOH (12 mL, 0.024 mol) was added to a stirred suspension of **11a** (2.3 g, 0.006 mol) in MeOH (50 mL) and the resulting mixture was stirred at 55 °C for 16 h. After solvent removal, the residue was diluted with water (5 mL), 25% HCl was added until neutrality and the resulting solid was filtered, washed with Et_2O, and dried under vacuum to give the title compound **12a** as a light yellow powder (2.3 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (br s, 1H), 10.99 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.56 (s, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.32 (br s, 4H), 2.44–2.51 (m, 4H), 2.27 (s, 3H); LC–MS (ESI) *m/z* 386 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{18}H_{19}N_5O_3S + H^+$ 386.1281, found 386.1285.

5.1.1.9. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (12b). By employment of the above-described procedure, starting from **11b**, compound **12b** was prepared. Yield, 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 10.76 (s, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.12 (s, 1H), 7.02 (d, *J* = 9.0 Hz, 2H), 3.82–3.70 (m, 4H), 3.48–3.20 (m, 4H); LC–MS (ESI) *m/z* 373 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{17}H_{16}N_4O_4S + H^+$ 373.0965, found 373.0970.

5.1.2. General procedure B

General procedure for the preparation of compounds **19**–**39** is illustrated below for the preparation of **38**.

5.1.2.1. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-1-phenyl-2-pyrrolidin-1-yl-ethyl)-amide (38). Ethyl chlorocarbonate (0.69 mL, 0.0072 mol) was slowly added to a mixture of **12b** (0.90 g, 0.0024 mol) and DIEA (4.14 mL, 0.024 mol) in dry DCM (30 mL)/DMF (18 mL) at −5 to 0 °C. The reaction was kept at the same temperature for 1 h then (*S*)-1-phenyl-2-pyrrolidin-1-yl-ethylamine dihydrochloride (3.17 g, 0.012 mol) was slowly added and the mixture was stirred at −5 °C for 0.5 h and at room temperature for 16 h. Next the mixture was diluted with dichloromethane and the organic layer washed with aqueous $NaHCO_3$ and brine before drying over sodium sulfate. Solvent was evaporated, and the residue was triturated with Et_2O and filtered to give 3.4 g of 3-(4-morpholin-4-yl-benzoylamino)-5-((*S*)-1-phenyl-2-pyrrolidin-1-yl-ethylcarbamoyl)-thieno[3,2-*c*]pyrazole, which was treated with MeOH (80 mL) and TEA (8 mL) and stirred at 50 °C for 6 h. The solvent was evaporated to dryness and the residue was purified by flash-chromatography (MeOH/DCM 1:6) to give the title compound **38** as a white solid (0.82 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 11.00 (s, 1H), 8.90 (br s, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.83 (s, 1H), 7.55–7.22 (m, 5H), 7.01 (d, *J* = 9.0 Hz, 2H), 5.30 (br s, 1H), 3.74 (m, 4H), 3.30–3.23 (m, 10H), 1.80 (m, 4H); LC–MS (ESI) *m/z* 545 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{29}H_{32}N_6O_3S + H^+$ 545.2329, found 545.2331.

By employment of the above-described procedure, starting from **12a** and using different amines, compounds **19–34** were prepared.

5.1.2.2. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5 carboxylic acid ethylamide (19). Yield, 45%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.03 (s, 1H), 8.54 (t, *J* = 5.6 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.66 (s, 1H), 7.10 (d, *J* = 8.8 Hz, 2H), 4.30–2.90 (m, 10H), 2.83 (s, 3H), 1.15 (t, *J* = 7.2 Hz, 3H); LC–MS (ESI) *m/z* 413 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{20}H_{24}N_6O_2S + H^+$ 413.1754, found 413.1752.

5.1.2.3. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid isopropylamide (20). Yield, 35%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 10.94 (s, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.70 (s, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 4.10 (m, 1H), 3.34 (m, 4H), 2.52 (m, 4H), 2.27 (s, 3H), 1.19 (d, *J* = 6.6 Hz, 6H); LC–MS (ESI) *m/z* 427 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{21}H_{26}N_6O_2S + H^+$ 427.1911, found 427.1914.

5.1.2.4. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid phenylamide (21). Yield, 30%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 11.01 (s, 1H), 10.25 (s, 1H), 8.00 (d, *J* = 9.0 Hz, 2H), 7.96 (s, 1H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.38 (m, 2H), 7.13 (m, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.33 (m, 4H), 2.52 (m, 4H), 2.25 (br s, 3H); LC–MS (ESI) *m/z* 461; $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{24}N_6O_2S + H^+$ 461.1754, found 461.1750.

5.1.2.5. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid benzylamide (22). Yield, 55%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H), 10.96 (s, 1H), 9.09 (t, *J* = 6.1 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.72 (s, 1H), 7.23–7.42 (m, 5H), 7.01 (d, *J* = 9.0 Hz, 2H), 4.49 (d, *J* = 6.1 Hz, 2H), 3.30 (m, 4H), 2.45 (m, 4H), 2.29 (br s, 3H); LC–MS (ESI) *m/z* 475

$[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{26}N_6O_2S + H^+$ 475.1911, found 475.1914.

5.1.2.6. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (1-phenyl-ethyl)-amide (23). Yield, 56%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 10.95 (s, 1H), 8.85 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.82 (s, 1H), 7.39–7.44 (m, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.25 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 5.15 (quin, *J* = 7.1 Hz, 1H), 3.31 (m, 4H), 2.46 (m, 4H), 2.24 (s, 3H), 1.51 (d, *J* = 7.1 Hz, 3H); LC–MS (ESI) *m/z* 489 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{28}N_6O_2S + H^+$ 489.2067, found 489.2070.

5.1.2.7. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (1-phenyl-propyl)-amide (24). Yield, 65%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 10.95 (s, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.83 (s, 1H), 7.38–7.45 (m, 2H), 7.35 (m, 2H), 7.25 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 4.83–4.94 (m, 1H), 3.34 (m, 4H), 2.47 (m, 4H), 2.25 (s, 3H), 1.72–1.96 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H); LC–MS (ESI) *m/z* 503 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}N_6O_2S + H^+$ 503.2224, found 503.2226.

5.1.2.8. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid benzhydryl-amide (25). Yield, 62%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 10.97 (s, 1H), 9.31 (d, *J* = 8.7 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.96 (s, 1H), 7.25–7.43 (m, 10H), 7.01 (d, *J* = 9.0 Hz, 2H), 6.40 (d, *J* = 8.7 Hz, 1H), 2.45–3.53 (m, 8H), 2.32 (br s, 3H); LC–MS (ESI) *m/z* 551 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{31}H_{30}N_6O_2S + H^+$ 551.2224, found 551.2223.

5.1.2.9. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (*R*)-1-phenyl-ethyl)-amide (26). Yield, 75%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.96 (s, 1H), 8.85 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.82 (s, 1H), 7.39–7.44 (m, 2H), 7.35 (m, 2H), 7.25 (m, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 7.40 (quin, *J* = 7.4 Hz, 1H), 2.99–3.55 (m, 4H), 2.40–2.82 (m, 4H), 2.32 (br s, 3H), 1.51 (d, *J* = 7.1 Hz, 3H); LC–MS (ESI) *m/z* 489 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{28}N_6O_2S + H^+$ 489.2067, found 489.2064.

5.1.2.10. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-1-phenyl-ethyl)-amide (27). Yield, 70%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 10.95 (s, 1H), 8.83 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 9.0 Hz, 2H), 7.81 (s, 1H), 7.37–7.42 (m, 2H), 7.34 (m, 2H), 7.20–7.27 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 5.14 (quin, *J* = 7.3 Hz, 1H), 3.16–3.45 (m, 4H), 2.55 (br s, 4H), 2.31 (br s, 3H), 1.49 (d, *J* = 7.1 Hz, 3H); LC–MS (ESI) *m/z* 489 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{31}H_{30}N_6O_2S + H^+$ 489.2067, found 489.2063.

5.1.2.11. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*R*)-1-phenyl-propyl)-amide (28). Yield, 55%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.95 (s, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.83 (s, 1H), 7.39–7.44 (m, 2H), 7.35 (m, 2H), 7.21–7.28 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 4.82–4.95 (m, 1H), 3.23–3.46 (m, 4H), 2.44–2.58 (m, 4H), 2.28 (br s, 3H), 1.73–1.95 (m, 2H), 0.93 (t, *J* = 7.32 Hz, 3H); LC–MS (ESI) *m/z* 503 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}N_6O_2S + H^+$ 503.2224, found 503.2227.

5.1.2.12. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-1-phenyl-propyl)-amide (29). Yield, 57%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.95 (s, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.83 (s, 1H), 7.38–7.43 (m, 2H), 7.35 (m, 2H), 7.22–7.28 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 4.83–4.96 (m, 1H), 3.19–3.51 (m, 4H), 2.41–2.72 (m, 4H), 2.28 (br s, 3H), 1.70–1.96 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H); LC–MS (ESI) *m/z* 503 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}N_6O_2S + H^+$ 503.2224, found 503.2221.

5.1.2.13. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (1-methyl-1-phenyl-ethyl)-amide (30). Yield, 30%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.94 (s, 1H), 8.46 (s, 1H), 7.97 (d, *J* = 9.0 Hz, 2H), 7.88 (s, 1H), 7.38–7.43 (m, 2H), 7.31 (m, 2H), 7.16–7.22 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 3.27–3.36 (m, 4H), 2.45–2.53 (m, 4H), 2.28 (br s, 3H), 1.69 (s, 6H); LC–MS (ESI) *m/z* 503 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}N_6O_2S + H^+$ 503.2224, found 503.2228.

5.1.2.14. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid indan-1-ylamide (31). Yield, 56%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 10.96 (s, 1H), 8.84 (d, *J* = 8.3 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.76 (s, 1H), 7.17–7.37 (m, 4H), 7.02 (d, *J* = 9.0 Hz, 2H), 5.54 (q, *J* = 8.1 Hz, 1H), 3.13–3.54 (m, 4H), 2.81–3.09 (m, 2H), 2.40–2.64 (m, 4H), 2.29 (br s, 3H), 1.88–2.07 (m, 2H); LC–MS (ESI) *m/z* 501 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}N_6O_2S + H^+$ 501.2067, found 501.2064.

5.1.2.15. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (1,2,3,4-tetrahydro-naphthalen-1-yl)-amide (32). Yield, 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 10.95 (s, 1H), 8.84 (d, *J* = 8.7 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 2H), 7.77 (s, 1H), 7.09–7.29 (m, 4H), 7.02 (d, *J* = 9.0 Hz, 2H), 5.14–5.30 (m, 1H), 3.14–3.53 (m, 4H), 2.71–2.91 (m, 2H), 2.40-2.63 (m, 4H), 2.28 (br s, 3H), 1.65–2.08 (m, 4H); LC–MS (ESI) *m/z* 515 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{28}H_{30}N_6O2S + H^+$ 515.2224, found 515.2226.

5.1.2.16. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-1-phenyl-2-pyrrolidin-1-yl-ethyl)-amide (33). Yield, 40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (br s, 1H), 10.97 (s, 1H), 8.84 (br s, 1H), 7.98 (d, *J* = 9.1 Hz, 2H), 7.83 (s, 1H), 7.42–7.49 (m, 2H), 7.22–7.40 (m, 3H), 7.00 (d, *J* = 9.1 Hz, 2H), 5.20 (br s, 1H), 3.14–3.53 (m, 4H), 3.5–2.2 (m, 6H), 2.40–2.63 (m, 4H), 2.26 (s, 3H), 1.73 (br s, 4H); LC–MS (ESI) *m/z* 558 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{30}H_{35}N_7O_2S + H^+$ 558.2646, found 558.2650.

5.1.2.17. 3-[4-(4-Methyl-piperazin-1-yl)-benzoylamino]-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-2-morpholin-4-yl-1-phenyl-ethyl)-amide (34). Yield, 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.96 (s, 1H), 8.78 (d, *J* = 8.3 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 7.81 (s, 1H), 7.41–7.46 (m, 2H), 7.36 (m, 2H), 7.23–7.29 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 5.15–5.32 (m, 1H), 3.47–3.65 (m, 4H), 3.15–3.45 (m, 4H), 2.34–2.91 (m, 10H), 2.27 (s, 3H); LC–MS (ESI) *m/z* 574 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{30}H_{35}N_7O_3S + H^+$ 574.2595, found 574.2599.

By employment of the above-described procedure, starting from **12b** and using different amines, compounds **35**–**37** and **39** were prepared.

5.1.2.18. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid benzylamide (35). Yield, 44%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 10.98 (s, 1H), 9.09 (t, *J* = 6.0 Hz, 1H), 8.00 (d, *J* = 9.0 Hz, 2H), 7.72 (s, 1H), 7.22–7.42 (m, 5H), 7.02 (d, *J* = 9.0 Hz, 2H), 4.49 (d, *J* = 6.0 Hz, 2H), 3.71–3.80 (m, 4H), 3.18–3.56 (m, 4H); LC–MS (ESI) *m/z* 462 $[M+H]^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{23}N_5O_3S + H^+$ 462.1594, found 462.1593.

5.1.2.19. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*R*)-1-phenyl-ethyl)-amide (36). Yield, 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (br s, 1H), 10.98 (s, 1H), 8.85 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 9.0 Hz, 2H), 7.82 (s, 1H), 7.39–7.44 (m, 2H), 7.36 (m, 2H), 7.22–7.28 (m, 1H), 7.02 (d, J = 9.0 Hz, 2H), 5.16 (quin, J = 7.2 Hz, 1H), 3.71–3.81 (m, 4H), 3.24–3.31 (m, 4H), 1.51 (d, J = 7.0 Hz, 3H); LC–MS (ESI) m/z 476 [M+H]$^+$; HRMS (ESI) m/z calcd for $C_{25}H_{25}N_5O_3S$ + H$^+$ 476.1751, found 476.1753.

5.1.2.20. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-1-phenyl-ethyl)-amide (37). Yield, 68%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (br s, 1H), 10.98 (s, 1H), 8.85 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 9.0 Hz, 2H), 7.82 (s, 1H), 7.39–7.44 (m, 2H), 7.36 (m, 2H), 7.22–7.28 (m, 1H), 7.02 (d, J = 9.0 Hz, 2H), 5.16 (quin, J = 7.2 Hz, 1H), 3.70–3.80 (m, 4H), 3.24–3.31 (m, 4H), 1.51 (d, J = 7.1 Hz, 3H); LC–MS (ESI) m/z 476 [M+H]$^+$; HRMS (ESI) m/z calcd for $C_{25}H_{25}N_5O_3S$ + H$^+$ 476.1751, found 476.1749.

5.1.2.21. 3-(4-Morpholin-4-yl-benzoylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid ((*S*)-2-morpholin-4-yl-1-phenyl-ethyl)-amide (39). Yield, 41%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 10.99 (s, 1H), 8.78 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 9.0 Hz, 2H), 7.82 (s, 1H), 7.41–7.48 (m, 2H), 7.36 (m, 2H), 7.24–7.30 (m, 1H), 7.02 (d, J = 9.0 Hz, 2H), 5.23 (m, 1H), 3.76 (m, 4H), 3.56 (m, 4H), 3.29 (m, 4H), 2.85 (dd, J = 12.6, 9.6 Hz 1H), 2.38–2.60 (m, 5H); LC–MS (ESI) m/z 561 [M+H]$^+$; HRMS (ESI) m/z calcd for $C_{29}H_{32}N_6O_4S$ + H$^+$ 561.2278, found 561.2277.

5.1.3. General procedure C

General procedure for the solid-phase synthesis of compounds **16** is illustrated below.

5.1.3.1. 3-(Acylamino)-1-polystyrene-trityl-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester (13). A solution of 3-(acylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester (**11**) (0.013 mol) in anhydrous DMF (20 mL) was added drop wise to a slurry of 9.9 g of polystyrene trityl chloride (Novabiochem 1.35 mmol/g loading) and DIPEA (6.9 mL, 0.040 mol) in anhydrous DCM (200 mL). The reaction mixture was gently stirred for 16 h at room temperature. The resin was isolated by filtration and washed with DMF, DCM and MeOH and dried under vacuum to give the title compound **13** (11.9 g with a nominal loading of 1.09 mmol/g).

5.1.3.2. 3-(Acylamino)-1-polystyrene-trityl-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid (14). 3-(Acylamino)-1-polystyrene-trityl-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid methyl ester (**13**) (11.7 g, 1.09 mmol/g, 0.013 mol) was added to a solution of 20% NaOH (52 mL, 0.32 mol), THF (120 mL) and MeOH (20 mL). The reaction mixture was shaken at room temperature for 72 h then the resin was isolated by filtration and washed with warm water, DMF, MeOH, Et_2O and dried under vacuum to give the title compound **14**.

5.1.3.3. *N*-Substituted-3-(acylamino)-1-polystyrene-trityl-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid amide (15). Hunig's base (0.275 mL, 0.0022 mol), TBTU (0.350 g, 0.0011 mol) and a 2 N solution of the amine in DMSO (0.550 mL) were added to a suspension of **14** (0.200 g, 1.09 mmol/g, 0.00022 mol) in anhydrous DCM (2 mL). The reaction mixture was shaken for 16 h at room temperature. The resin was isolated by filtration and washed sequentially with DCM, MeOH and dried under a current of nitrogen to give the resin bound protected thienopyrazole amide (**15**).

5.1.3.4. *N*-Substituted-3-(acylamino)-1*H*-thieno[3,2-*c*]pyrazole-5-carboxylic acid amide (16). 10% TFA in DCM (2 mL) was added to 0.200 g of **14** in each Miniblock vessel. The red suspension was shaken for 1 h then filtered and the resin washed twice with MeOH (1 mL), DCM, (1 mL), MeOH (1 mL). The filtered solution was evaporated under reduced pressure by EZ-2 (Genevac) to give a crude solid or oil, which was purified by preparative HPLC to give 1*H*-thieno[3,2-*c*]pyrazoles derivatives as single compounds (**16**).

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Supplementary data

Elemental analysis data for compounds **19**, **22**–**32**, **34**, **36**, **38**, **39**, selectivity data for compound **38**, and crystallographic methods. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.048. Aurora assay, cell proliferation assay, cell cycle analysis by flow cytometry, and Western blot analysis are available at http://pubs.acs.org/doi/abs/10.1021/jm049076m.

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